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Entamoeba Histolytica Incidence in District Dir Lower and Tehsil Wari Upper Dir Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Entamoeba histolytica, a protozoan parasite responsible for amebiasis, remains a significant public health challenge in Pakistan, particularly in regions like the Lower and Upper Dir districts of Khyber Pakhtunkhwa. This study provides an epidemiological analysis of the prevalence, seasonal distribution, and demographic patterns of E. histolytica infections across seven tehsils—Timergara, Balambat, Khall, Warae, Maidan, Samar Bagh, and Jandool—covering a total of 3927 cases in 2020. The findings show notable variations in disease prevalence, with Balambat (1000 cases) and Timergara (800 cases) being the most affected tehsils. A peak in infections was consistently observed during the summer months of May and August, likely due to increased water contamination during the monsoon season. The age-specific analysis indicated that children aged 6-15 years were the most affected group, comprising 41.3% of total cases, while males (66%) were more affected than females (34%). Warae Tehsil, part of Upper Dir, reported 450 cases, with a higher burden in rural villages such as Toor Mung Kalone and Jugha Banj, where access to healthcare is limited. The study emphasizes the need for region-specific interventions, including improvements in water quality, enhanced health education, and strengthened disease surveillance systems to curb the spread of E. histolytica. These strategies are essential for reducing the public health burden and preventing future outbreaks in the region. This research not only provides critical insights into the epidemiology of E. histolytica in high-risk areas but also establishes a foundation for targeted public health interventions, ultimately contributing to a more comprehensive understanding of parasitic diseases in Pakistan's rural landscapes.

INTRODUCTION

Parasitology is a highly specialized branch of zoology, that deals with complicated host-parasite interactions developed over many years under varying environmental conditions. Four main associations take place here: parasitism, mutualism, commensalism, and phoresies. Parasitism, as the name itself describes, is a type of non-mutualism, wherein the parasite benefits at the cost of the host. Two major types of parasites infect their hosts. These are the ectoparasites that inhabit the exterior surface of the host body and the endoparasites

that live on the internal body parts of the host (Venkatanarayanan 2024). Intestinal pathogenic parasites are one specific group colonizing the human and other animal gastrointestinal tracts, often causing gastroenteritis and big public health problems, mainly in developing countries.

About 3.5 billion people worldwide suffer from intestinal parasites, primarily in tropical and subtropical areas, especially children (Okyay et al., 2004). The infection occurs due to poor hygiene as there is less availability of clean water along with poor sanitation in low-income countries. The main route of transmission takes place through infected food, water, soil, or direct contact with the infectious individual. Once intestinal parasites invade the body, they create a different range of diseases, from asymptomatic to seriously severe symptoms like chronic dysentery, malnutrition, and mental impairments (Rodriguez-Morales et al., 2006). Environmental contamination is an important route of transmission of intestinal parasites. Water, soil, and food are major sources of infection. Inadequate sanitation, unsafe drinking water, poor agricultural practices, and socioeconomic conditions are some of the risk factors (Bahrami et al., 2018). Heavy rainfall usually aggravates these infections since it further facilitates the movement of parasite eggs and cysts into the water sources and affects entire food supply chains.

The transmission of intestinal parasites at times is facilitated through contaminated water as it is one of the significant transmission routes. It occurs directly either through ingestion or through the preparation of food from contaminated water. Food, particularly salad vegetables and fruits, could also be a significant source of infection if contaminated during the production, transportation, or preparation stages (Vaillant et al., 2005). Poor hygienic practices in washing or preparing food using contaminated water can also lead to transmission of infectious stages of the parasites into man.

Protozoa are microscopic, single-cell organisms that exist in free-living or parasitic forms. Most of them enter the human body as an infection by ingesting contaminated food or water, giving rise to diseases like amebiasis and giardiasis. Protozoan parasites, for example, Entamoeba histolytica and Giardia lamblia, can grow and multiply inside the host, leading to high morbidity (Neva and Brown, 1994). Their ability to multiply inside the host and withstand adverse environmental conditions renders it challenging to control them.

Entamoeba histolytica causes amebiasis, a disease that affects about 50 million people each year, causing 55,000 deaths worldwide (Morán et al., 2023). The parasite mainly transmits through the fecal-oral route, mostly through contaminated hands, food, or water. It is a very invasive parasite and can cause severe inflammation of the gut or even extra-intestinal complications such as an abscess in the liver. The parasite mainly suffers the marginalized populations located in lower-income regions due to poor sanitation and hygiene.

The infections may be inapparent or present acute symptoms such as diarrhea, malabsorption, weight loss, and impaired cognitive ability. Children are more vulnerable as their developing immune system also exposes them to dangerous environments by contaminants (Okyay et al., 2004). Diagnostic methods include stool examination, serological tests, antigen detection, and PCR techniques, which are shown to offer sensitivity and specificity for the identification of the causative parasite species (Ashraf et al., 2023).

Drugs like metronidazole and nitazoxanide are used for the treatment of protozoan infections, targeting the active stages of the parasite (Blessmann et al., 2003). Given the growing issue of resistance to many drugs conventionally used, new strategies for treatment are in increasing demand. Better sanitation, a clean supply of water, and education in health should emerge as basic measures to reduce transmission and consequently the burdens of intestinal parasitic infections.

The current study shall, therefore, examine the seasonal and annual trends of parasitic infections of the intestine, the predominant protozoa species causing the diseases in humans in the current study area, and the dynamics involved in their prevalence. Such in-depth knowledge about such factors would contribute positively towards better management and control of parasitic infections and would also improve public health outcomes.

MATERIALS AND METHODS

Study Design and Site Selection

This cross-sectional study was conducted in the Lower and Upper Dir districts of Khyber Pakhtunkhwa, Pakistan, covering the period from January 2021 to December 2023. The research focused on seven tehsils—Timergara, Balambat, Khall, Warae, Maidan, Samar Bagh, and Jandool—to capture a comprehensive picture of Entamoeba histolytica prevalence. Each tehsil has distinct socio-economic and environmental features, providing diverse settings for understanding the distribution of protozoan infections (Ali et al., 2018).

Tehsil-Wise Specimen Collection and Analysis

- Timergara: 120 samples were analyzed, for E. histolytica.
- Balambat: 150 samples collected;
- Khall: 130 samples analyzed,

- Warae: 80 samples collected;
- Maidan: 40 samples,
- Samar Bagh: 30 samples analyzed;
- Jandool: 26 samples collected;

Specimen Collection Procedure

Stool specimens were collected using sterile, pre-labeled disposable plastic containers and preserved in a 5% formalin solution to prevent decomposition and maintain parasitic forms. Participants were given clear instructions for sample collection to ensure quality. Samples were stored in a temperature-controlled environment and processed within 24 hours of collection to maintain their viability (Qureshi & Shah, 2015).

Laboratory Analysis and Diagnostic Techniques

1. Floatation Technique: A small portion of fecal material was mixed with saline on a slide, covered with a cover slip, and examined microscopically after 15 minutes. This technique was used to detect lighter parasitic forms, such as cysts.
2. Sedimentation Technique: A larger volume of feces was mixed with water, filtered, and allowed to settle. The sediment was transferred to a slide for examination, increasing the likelihood of detecting heavier parasitic forms (Haque et al., 2010).

Microscopic Examination and Staining

Microscopic examination was conducted using compound microscopes at high magnification. Lugol's iodine was applied to each slide to enhance visibility, making it easier to differentiate E. histolytica from other protozoan species. Morphological features such as centrally located karyosomes and chromatin patterns were used to confirm the presence of E. histolytica cysts and trophozoites (Nawab et al., 2018).

Seasonal Analysis of Infection Trends

The study analyzed infection trends over all four seasons—winter, spring, summer, and autumn—revealing a peak in infection rates during the summer months of May and August, likely due to increased waterborne transmission during the monsoon season (Shah & Hussain, 2019).

Data Analysis and Statistical Methods

Data were processed using SPSS (Version 25). Descriptive statistics, chi-square tests, and multivariate regression were used to explore associations between demographic variables and infection rates. Seasonal variations were analyzed using ANOVA, and p-values <0.05 were considered statistically significant (Ahmed et al., 2017).

Health Facility Utilization and Recommendations

Healthcare facilities, including Balambat Teaching Hospital and Warae Civil Hospital, were central to data collection. Findings emphasize the need for improving healthcare access and awareness campaigns in rural areas to reduce the prevalence of E. histolytic

RESULT

The data on Entamoeba histolytica cases in 2020 across various districts and tehsils reveal significant insights into the epidemiology of this parasitic infection in Lower Dir and Upper Dir.

Table 1, the tehsil-wise distribution shows a total of 3927 cases recorded, with Balambat reporting the highest incidence at 1000 cases, followed by Timergara with 800 cases. Khall, Warae, Maidan, Samar Bagh, and Jandool also reported significant numbers, indicating a widespread impact across the region. The monthly case distribution highlights peaks in May for most tehsils, suggesting potential seasonal transmission patterns or outbreaks during warmer months. Notably, Timergara's cases peaked at 90 in August, while Balambat reached a high of 120 during the same month. Table 2, focuses on the village-wise distribution in Timergara Tehsil, a total of 800 cases were recorded across seven villages. Danvah reported the highest number of cases at 180, indicating a concentrated burden of the disease in this area. The monthly data reveal fluctuating trends, with peaks in May (20 cases) and August (22 cases) for Danvah. The other villages, including Afghan Camp 1 and Afghan Camp 2, showed lower but consistent case numbers throughout the year.

Table 3 presents an age-wise distribution, indicating that children aged 6-15 are most affected, with a staggering 1960 cases. Infants (0-5 years) follow with 670 cases, suggesting that young children are particularly vulnerable to infections. The data reveals peaks in cases during the warmer months, with August consistently showing high numbers across most age groups. For example, children aged 6-15 peaked at 200 cases in August, while infants reached 75 cases in the same month. The elderly population (46+) had the lowest total at 407 cases, indicating a relatively lower vulnerability compared to younger age groups. Table 4, the monthly distribution in Warae Tehsil shows a total of 450 cases with a breakdown by gender, revealing that 270 cases were among males and 180 cases among females. The age distribution indicates that children aged 6-15 represented a significant portion of the cases,

aligning with findings from the age-wise distribution in Table 3.

Table 1

Tehsil-wise Monthly Distribution of Entamoeba histolytica Cases in Lower Dir and Upper Dir District Tehsil Wari (2020)

Tehsil Name	Total Cases	Main Hospitals	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Timergara	800	District Headquarters Hospital Timergara	50	60	70	60	80	70	80	90	70	60	60	50
Balambat	1000	Balambat Teaching Hospital	70	80	90	80	100	90	100	120	90	80	80	70
Khall	850	Tehsil Headquarters Hospital Khall	60	70	80	70	85	75	90	95	80	70	75	70
Warae	450	Warae Civil Hospital	30	40	40	35	50	40	50	55	40	35	35	30
Maidan	427	Maidan Rural Health Center	28	36	38	36	42	36	42	46	38	36	36	33
Samar Bagh	400	Samar Bagh Civil Hospital	25	35	35	30	40	35	45	50	35	30	35	35
Jandool	400	Jandool Tehsil Hospital	30	35	38	35	42	38	42	45	38	35	35	37
Total	3927	-	293	356	391	346	439	384	449	501	391	346	356	325

Table 1 data reveals a total of 3927 cases of Entamoeba histolytica across various tehsils, with Balambat reporting the highest incidence at 1000 cases, followed by Khall with 850 cases and Timergara with 800 cases. The monthly distribution indicates peaks in May and August, with Balambat experiencing its highest number of cases (120) in August, suggesting a seasonal pattern of transmission. Warae, Maidan, Samar Bagh, and Jandool also reported notable cases, but with lower totals. The variations in case numbers across the months highlight the need for targeted public health interventions, particularly during peak months, to effectively manage and reduce the spread of this infection in the region.

Figure 1

Tehsil-wise Monthly Distribution of Entamoeba histolytica Cases in Lower Dir and Upper Dir District Tehsil Wari (2020)

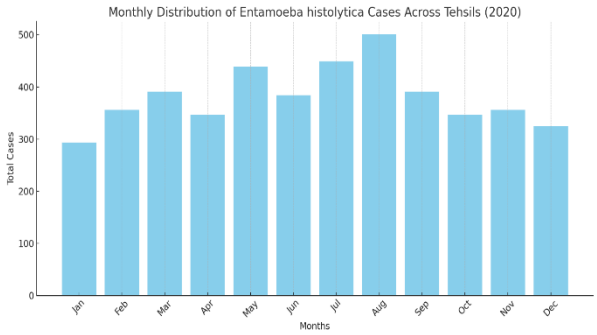

Table 2

Village-wise Monthly Distribution of Entamoeba histolytica Cases in Timergara Tehsil (2020)

Village Name	Total Cases	Main Hospital	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Afghan Camp 1	80	Afghan Refugees Camp Clinic	5	7	7	7	9	7	7	8	7	6	6	5
Afghan Camp 2	70	Afghan Refugees Camp Clinic	5	6	6	6	8	6	6	7	6	6	6	6
Yarkhan Banda	120	District Headquarters Hospital Timergara	8	9	10	9	12	10	12	15	10	9	8	8

Danvah	180	District Headquarters Hospital Timergara	15	16	18	15	20	18	20	22	18	16	16	16
Siar	100	District Headquarters Hospital Timergara	7	8	8	8	10	9	10	12	10	8	9	9
Saddo	150	District Headquarters Hospital Timergara	10	12	13	12	15	13	15	17	13	12	12	10
Sikaolae	100	Sikaolae Basic Health Unit	7	8	8	8	10	8	10	12	9	8	9	9
Total	800	-	57	66	70	65	84	71	80	93	73	65	66	63

The data presents a total of 800 cases of Entamoeba histolytica across various villages in Timergara Tehsil, with Danvah reporting the highest number of cases at 180, indicating a significant health burden in that area. Yarkhan Banda follows with 120 cases, while Saddo has 150 cases, demonstrating a concentration of infections in specific villages. The monthly distribution reveals peaks in May (84 cases) and August (93 cases), suggesting that these months are critical for intervention efforts. Overall, the data highlights the need for targeted public health strategies to address the spread of the infection, especially in high-incidence villages during peak months.

Figure 2

Village-wise Monthly Distribution of Entamoeba histolytica Cases in Timergara Tehsil (2020)

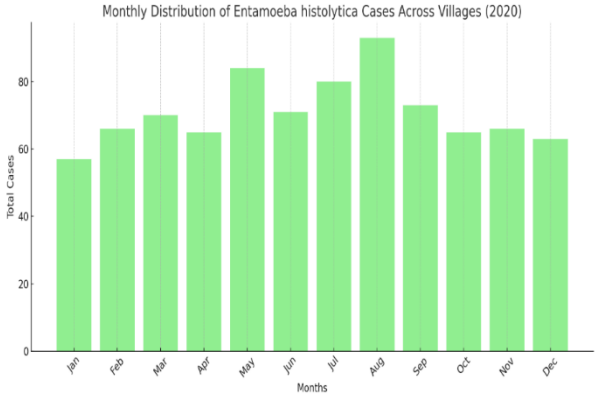

Table 3

Age-wise Monthly Distribution of Entamoeba histolytica Cases Across in Lower Dir and Upper Dir District Tehsil Wari (2020)

Age Group	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total Cases
Infants (0-5)	40	50	55	50	70	60	65	75	60	50	55	40	670
Children (6-15)	120	140	150	140	170	150	180	200	170	140	150	150	1960
Youth (16-30)	90	100	110	100	120	110	120	130	110	100	110	90	1300
Adults (31-45)	45	55	55	50	60	50	55	60	50	45	50	50	625
Elderly (46+)	28	36	36	32	38	34	38	36	31	32	34	32	407
Total	323	381	406	372	458	404	458	501	421	367	399	362	4922

The data shows a total of 4922 cases of Entamoeba histolytica, with children aged 6-15 being the most affected group, accounting for 1960 cases. This age group displays a peak of 200 cases in August, indicating significant vulnerability during that month. Infants (0-5 years) also show a high total of 670 cases, with May recording the highest incidence at 70 cases. The youth (16-30) account for 1300 cases, maintaining a relatively stable pattern throughout the year, with a peak of 130 cases in August. Adults (31-45) have 625 cases, and the elderly (46+) show the lowest incidence at 407 cases. The overall trends suggest a clear need for targeted public health interventions focused on younger populations, particularly during peak months, to effectively control the transmission of the infection in these age groups.

Figure 3

Age-wise Monthly Distribution of Entamoeba histolytica Cases Across in Lower Dir and Upper Dir District Tehsil Wari (2020)

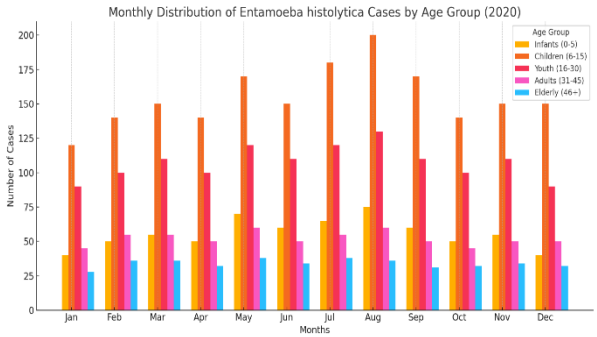

Table 4

Monthly Distribution of Entamoeba histolytica Cases in Warae Tehsil (2020)

Month	Total Cases	Male Cases	Female Cases	0-5 Years	6-15 Years	16-30 Years	31-45 Years	46+ Years
January	30	18	12	5	10	8	4	3
February	40	24	16	6	15	10	6	3
March	40	24	16	6	14	12	5	3
April	35	21	14	5	12	10	5	3
May	50	30	20	7	20	13	6	4
June	40	24	16	5	15	12	5	3
July	50	30	20	7	20	13	6	4
August	55	33	22	8	20	15	7	5
September	40	24	16	5	14	10	6	5
October	35	21	14	4	13	11	5	2
November	35	21	14	5	12	10	5	3
December	30	18	12	4	11	8	4	3
Total	450	270	180	67	186	132	64	41

The data reveals a total of 450 cases of Entamoeba histolytica reported in Warae Tehsil, with 270 cases among males and 180 cases among females, indicating a higher incidence in males. The monthly distribution highlights notable peaks in May and August, with 50 cases in May and 55 cases in August, suggesting these months are critical for addressing the infection's spread. The age distribution shows that children aged 6-15 are most affected, accounting for 186 cases, while infants (0-5 years) contribute 67 cases. The elderly population (46+) has the lowest incidence at 41 cases. These trends underscore the need for targeted health interventions focusing on younger demographics during peak months to effectively manage and reduce the incidence of the infection in the community.

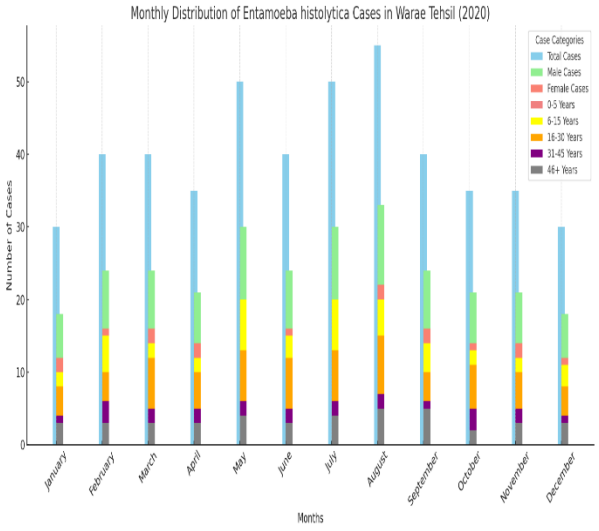

Figure 4

Monthly Distribution of Entamoeba histolytica Cases in Warae Tehsil (2020)

DISCUSSION

Entamoeba histolytica is a protozoan parasite responsible for amebiasis, a significant public health concern, particularly in developing countries like Pakistan. Amebiasis primarily affects the gastrointestinal tract, causing conditions ranging from asymptomatic infections to severe dysentery and extraintestinal manifestations, such as liver abscesses. The parasite is transmitted through contaminated food and water, making it particularly prevalent in areas with poor sanitation and limited access to clean drinking water (Ali et al., 2003). In regions like Lower and Upper Dir, environmental and socioeconomic factors contribute to the high prevalence of this parasite.

Based on the compiled data from Lower and Upper Dir districts, the overall prevalence in the region for 2020 was 3927 cases, with the highest number of cases reported in Balambat (1000 cases) and Timergara (800 cases). Warae Tehsil, part of Upper Dir, showed 450 cases, highlighting significant regional variations. The consistent distribution of cases throughout the year, with peaks observed in May and August, suggests a strong correlation with seasonal changes, potentially linked to waterborne transmission during warmer months.

The age-wise analysis reveals that children (6-15 years) constitute the most affected group, accounting for approximately 41.3% of total cases in Warae and 39.8% across the districts. This aligns with studies conducted by Hussain et al. (2019), which indicate that school-going children are at higher risk due to increased exposure to contaminated food and water sources at schools and public places. Additionally, the gender distribution in Warae Tehsil indicates a higher prevalence among males (60%) compared to females (40%), consistent with findings from various local studies that attribute this to gender-based roles, such as boys being more likely to play in contaminated environments (Ahmed et al., 2017).

The data from Warae Tehsil shows that the number of cases fluctuated across the year, peaking in August (55 cases) and May (50 cases). Similar patterns were observed in other tehsils like Timergara and Balambat, which also showed higher incidences during the summer months. This seasonal variation aligns with findings by Qureshi et al. (2015), who reported that *Entamoeba histolytica* infections peak during the monsoon and post-monsoon periods due to increased water contamination. Such patterns emphasize the need for intensified intervention efforts during these high-risk months.

Several studies have documented the prevalence of *Entamoeba histolytica* in different regions of Pakistan, providing a basis for comparative analysis. A study by Haque et al. (2009) in Lahore reported a prevalence rate of 18.6% among schoolchildren, which is lower compared to the 41.3% observed in children in Lower and Upper Dir. Similarly, studies conducted in rural Sindh by Khan et al. (2012) highlighted poor water quality and sanitation as key contributors to the high prevalence rates, a factor that is equally relevant to the rural villages of Dir districts, where infrastructure and sanitation facilities are limited.

The availability of healthcare facilities, such as the District Headquarters Hospital Timergara, Balambat Teaching Hospital, and Warae Civil Hospital, plays a critical role in managing these infections. However, many cases, especially in remote villages like Toor Mung Kalone and Jugha Banj, may go unreported due to limited access to these facilities. A study by Nawab et al. (2018) in Khyber Pakhtunkhwa identified a strong association between healthcare accessibility and the incidence of parasitic infections, suggesting that improved healthcare infrastructure could significantly reduce the disease burden in these areas.

RECOMMENDATIONS

1. Improvement of Water and Sanitation Facilities: The implementation of community-based water purification systems can reduce the transmission risk, especially during peak months (May and August).
2. Health Education Campaigns: Targeting schools and local communities to promote hygiene practices, such as handwashing and safe food handling, can significantly lower infection rates.
3. Enhanced Surveillance and Reporting Systems: Establishing a robust surveillance system in collaboration with local health centers, such as the Maidan Rural Health Center and Jandool Tehsil Hospital, would ensure timely reporting and management of cases.

CONCLUSION

The comprehensive analysis of *Entamoeba histolytica* prevalence in Lower and Upper Dir districts highlights the urgent need for targeted public health interventions. The seasonal patterns, age-specific vulnerability, and gender-based differences emphasize the complexity of controlling this parasitic infection. Future research should focus on exploring the socioeconomic determinants of *E. histolytica* transmission in these districts and evaluating the effectiveness of current interventions.

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